

Stability analysis of a model gene network links aging, stress resistance, and negligible senescence

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Several animal species are considered to exhibit what is called negligible senescence, i.e. they do not show signs of functional decline or any increase of mortality with age, and do not have measurable reductions in reproductive capacity with age. Recent studies in Naked Mole Rat (NMR) and long-lived sea urchin showed that the level of gene expression changes with age is lower than in other organisms. These phenotypic observations correlate well with exceptional endurance of NMR tissues to various genotoxic stresses. Therefore, the lifelong transcriptional stability of an organism may be a key determinant of longevity. However, the exact relation between genetic network stability, stress-resistance and aging has not been defined. We analyze the stability of a simple genetic-network model of a living organism under the influence of external and endogenous factors. We demonstrate that under most common circumstances a gene network is inherently unstable and suffers from exponential accumulation of gene-regulation deviations leading to death. However, should the repair systems be sufficiently effective, the gene network can stabilize so that gene damage remains constrained along with mortality of the organism, which may then enjoy a remarkable degree of stability over very long times. We clarify the relation between stress-resistance and aging and suggest that stabilization of the genetic network may provide a mathematical explanation of the Gompertz equation describing the relationship between age and mortality in many species, and of the apparently negligible senescence observed in exceptionally long-lived animals. The model may support a range of applications, such as systematic searches for therapeutics to extend lifespan and healthspan.

The Naked Mole Rat is an example from a growing list of animal species with no signs of aging or reduction in reproductive capacity with age. On the other hand, the age-dependent increase in death rate for most species, including humans, follows the Gompertz equation, which describes an exponential increase in mortality with age. In this work we construct a mathematical model of a gene network, described by a few differential equations with unstable and stable solutions, corresponding to normal and “negligible” senescence. For this purpose, we define aging as an exponential accumulation of epigenetic dysregulation errors, and show that the Gompertz “law” is a direct consequence of the inherent genetic instability of the network. On the organism level this leads to the loss of stress resistance, the onset of age-related diseases, and finally to death. We suggest and analyze several strategies for gene network stabilization, which can be exploited for future life-extending therapeutics.

Introduction

Aging in most species studied, including humans, leads to an exponential increase of mortality with age, primarily from a variety of age-related diseases. A growing number of animal species are recognized to exhibit what is called negligible senescence, i.e. they do not show mea-

surable reductions with age, in their reproductive ability or functional capacities [1]. Death rates in negligibly senescent animals do not increase with age as they do in senescent organisms. One negligibly senescent species is the ocean quahog clam, which lives about 400 years in the wild [2] and is the longest-living non-colonial animal. Its extreme longevity is associated with increased resistance to oxidative stress in comparison with short-lived clams [3]. No noticeable signs of aging were found in a few turtle species, such as Blanding’s turtle, whose lifespan is over 75 years [4], and the painted turtle, which was documented to live at least 61 years. Studies showed that these turtles increase offspring quality with age, so they are considered to be negligibly senescent [5]. The archetypical example of negligible senescence is the Naked Mole Rat (NMR), which has been documented to live in captivity for as long as 28 years [6] with no signs of increasing mortality, little or no age-related decline in physiological functions, sustained reproductive capacity, and no reported instances of cancer throughout their long lives [7]. These phenotypic observations correlate well with exceptional resistance of NMR tissues to diverse genotoxic stresses [8, 9]. Even more examples of negligibly senescent organisms may be found in the AnAge database [10].

In contrast, aging in most species studied, including humans, follows the Gompertz equation [11] describing

any single protein and here represents a simple measure of the overall connectivity of the genetic network (See the section below on Strategies for stabilization of genetic networks). The constant c reflects the combined efficiency of proteolysis and heat shock response systems, mediating degradation and refolding of misfolded proteins, respectively, whereas δ characterizes the DNA repair rate. Furthermore, the model includes the force terms, $f_p(t)$ and $f_g(t)$, which characterize the proteome and genome damage rates, respectively. The “forces” can represent any of a number of things, including oxidative stress (metabolic), temperature, gamma-radiation (environmental), that are imperfectly compensated by protective mechanisms.

Eqs. (1) and (2) can only hold in their simple linearized form if defects do not interfere with the repair machinery or any other rare essential subsystems of the gene network. As we will see below if, for example, a defect alters a DNA repair system-associated gene or protein, the repair rate drops, the system becomes unstable and may quickly diverge from its normal state [15, 16]. To avoid the complications from introducing such nonlinearities, we adopt a simple hypothesis as to how defects in the evolving gene network could be responsible for the demise of a cell or organism. Specifically, we assume that mortality at any time is dependent on the probability of a defect to “land” on and damage or dysregulate an essential gene. Because any gene in the model can be dysregulated for a short time (brief relative to lifespan) and then “repaired”, a gene is considered essential if the disruption of its expression, even for a limited time, is lethal to the cell in which it was disrupted. The suggested picture is quite general, however, and is easily extended to multicellular/metazoan animals, since the stability boundaries are the same – only the exponents will be reduced because some threshold fraction of cells must die (in some most-vulnerable tissue compartment) to produce organism lethality. In this case the population dynamics of a set of gene networks representing $N(t)$ organisms can be represented by:

$$\dot{N}(t) = -M(t)N(t), \quad (3)$$

where $M(t) = \omega e_g/G$ is the mortality rate, proportional to the fraction of mis-regulated genes, e_g/G , and ω is an empirical factor, roughly a measure of the (small) fraction of genes in the whole genome that are essential. The presented model is, obviously, an extreme simplification. Nevertheless it can be rigorously derived as a limiting case for the dynamics of a more complex genetic regulatory network, where the number of dysregulated genes is sufficiently large [17].

Since aging is a slow process, Eqs. (1)-(3) can be further simplified by neglecting second derivatives with respect to time,

$$\dot{M}(t) = \Lambda M(t) + \omega F(t), \quad (4)$$

where $F = f/(c + \delta)$ is a combined measure of genotoxic stress, $f = \beta K f_p + c f_g + \dot{f}_g$, and $\Lambda = (\beta p G K - c \delta)/(c + \delta)$

is the exponent characterizing genetic-network stability, which is precisely the propagation rate of gene-expression-level perturbations. In the long run, stress levels can be averaged out and presumed to be time-independent, $f_{p,g}(t) = \text{const}$, which yields the following expression for the age-dependent mortality rate:

$$M(t) = \frac{\omega F}{\Lambda} (\exp \Lambda t - 1). \quad (5)$$

The nature of the solution is very different depending on the sign of the exponent Λ . Whenever the combined efficiency of all repair systems is lower than a measure of the defect proliferation rate,

$$R_0 = \frac{\beta p G}{c \delta} K > 1, \quad (6)$$

the gene network becomes inherently unstable, $\Lambda > 0$, and the number of regulatory errors (defects) grows exponentially, $M(t) \sim \exp(\Lambda t)$, which is identical to the well-known Gompertz law [11]. The average lifespan does not depend on the initial population size,

$$t_{le} \approx \frac{1}{\Lambda} \ln \left(1 + \frac{1}{\gamma} + \sqrt{\frac{\pi}{2\gamma}} \right), \quad (7)$$

but depends on both the exponent Λ and on the genotoxic stress level through the parameter $\gamma = \omega F/\Lambda^2$, which is typically very small. Therefore in the limit, when the life expectancy is large, the average lifespan can be approximated by $t_{le} \approx \Lambda^{-1} \log(1/\gamma)$.

A considerably more intriguing situation occurs when the genome is stable, $\Lambda < 0$ ($R_0 < 1$), and the gene network may remain stable under reasonable stress conditions for a very long time. The fractions of dysregulated genes and of misexpressed proteins will then stabilize at constant levels, as will the mortality rate itself, $M_\infty = \omega F/|\Lambda|$. Constant mortality means that the population of animals dies off exponentially rather than age-dependently: $N(t) = N_0 \exp(-M_\infty t)$, which is much slower than the prediction from the Gompertz law. We believe that the age-independent mortality observed in NMR experiments over a very long lifespan [6], together with exceptional stress resistance of NMR tissues [8], may be manifestations of this stable scenario. We predict that the gene networks of negligibly senescent animals are exceptionally robust, and the number of dysregulated genes will scarcely change with age. This argument is supported by the observations of [14], in which the number of genes differentially expressed with age was compared between NMR, mice and humans.

Aging in a transcriptome of fruit flies

The model summarized by Eqs. (3) and (4) predicts that the number of gene regulatory errors will most often grow exponentially as animals age, as will the risk

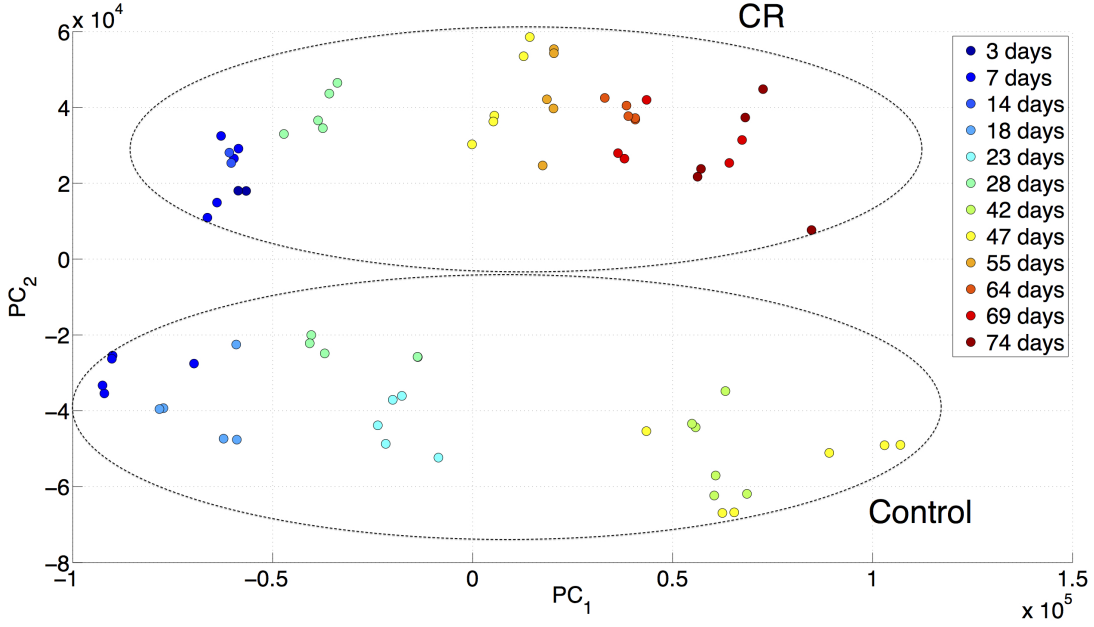


Figure 2: Principal components analysis of gene expression profiles in aging flies (data from [18]), fed on control (ad lib) and calorically restricted diets. Every point represents a transcriptome for flies of a specific age and diet. As the animals age, the genetic network accumulates regulation errors and the transcription levels change in a single direction, up to a limit beyond which viability cannot be maintained.

of death (mortality). This means that gene expression levels (or metabolite levels, etc.) should change with age and deviate from their healthy/youthful states. To test this prediction, we reanalyzed gene expression of fruitflies from the data of reference [18]. The measurements were performed at 6 adult ages, for two groups of *Drosophila melanogaster*: normally (“ad lib”) fed control flies and calorically restricted (CR) flies. Figure 2 is a Principal Components (PC) analysis plot, in which each point represents the state of gene expression for one combination of age and diet. The first/horizontal principal component, PC1, is the variance of an empirically derived cluster of 19 genes, which strongly correlates with the age of adult animals in either of the two diet groups. Points on the extreme left correspond to the youngest flies, and points for older age-groups are displaced progressively to the right.

Thus, deviation of the gene expression profile from the healthy young state increases with age, indicating that the number and extent of dysregulated genes increases along with mortality. However, no flies in either diet group were able to survive to the right of a certain boundary, beyond which the accumulation of gene-expression abnormalities becomes incompatible with survival of the organism.

Interestingly, the variance along the second principal component, PC2, creates a distinct separation between the ad lib (control) and CR-fed flies, based on expression variance in a different subset of 18 genes. This, of course, cannot be explained with the help of our basic

model, but the proper generalization of that model is described in [17]. It is of great interest, nevertheless, that the entire range of variation in the PC1 dimension was less for CR flies than for control flies, despite spanning a much greater range of ages.

Genetic-network stability and stress resistance

Extreme longevity has long been associated with exceptional resistance to a variety of stresses. And conversely, the decrease of stress resistance with age is one of the best-established indices of aging. These phenomena can be explained using our simplified model. To apply the model to experimental data, we reanalyzed data from reference [19], in which flies of varying age were exposed to radiation during an exposure time $T_1 \sim 0.1 \text{ day}$ and the median lethal dose LD_{50} was measured, corresponding to 50% lethality of flies over a subsequent period of $T = 2 \text{ days}$. To model the experiment, we assumed that the animals were subjected to an external genotoxic stress at age $t = t_0$ for a duration T that is small compared to the lifespan of the animals. We solve Eqs. (3), (4) and show that for “normally” aging animals, in which $\Lambda > 0$, the dose required to produce 50% mortality decreases exponentially with age:

$$D_{50} \sim \frac{\ln 2}{\omega T} - \frac{F_0}{\Lambda} \exp(\Lambda t_0), \quad (8)$$

in qualitative agreement with the experimental data [19]. We interpret this to mean that stress resistance of “normally” aging animals decreases progressively with age, and the average lifespan roughly corresponds to the age at which endogenous (or common exogenous) stresses alone are sufficient to cause death. At late ages, $\Lambda t_0 \gtrsim 1$, the total accumulated error load, $e_g(t)$, becomes so large that the linear model defined by Eq. (3) and (4) fails. A more comprehensive analysis shows that LD_{50} remains positive, but shrinks exponentially at the most advanced ages [17]. Eq.(8) also implies that measures of stress resistance should constitute robust biomarkers of aging.

We suggest that negligibly senescent organisms correspond to $\Lambda < 0$. That situation can be achieved only if the repair systems efficacies are relatively strong. Moreover, the model predicts no further decrease with age in the lethal-stress dosage LD_{50} beyond a threshold age, $|\Lambda|t_0 \gtrsim 1$. Both predictions are well supported by experimental data. It is known that negligibly senescent organisms are more stress resistant than shorter-lived ones [8]. For example, a comparison of survival between negligibly senescent vs. short-lived clam tissues treated with tert-butyl hydroperoxide showed much higher resistance to oxidative stress in long-lived clams [3]. Also Oxygen Radical Absorbance Capacity (ORAC) was measured in young and old clams of both types. The experiment showed an age-related decline in ORAC for shorter-lived clams, whereas ORAC did not change with age in tissues of negligibly-senescent clams, which is exactly what the model predicts for a negligibly-senescent animal.

The proposed model may be considered as a general theory that subsumes previous “error catastrophe” theories [15, 16] as special cases. It was long considered that error catastrophes had been disproved by the following experiment [20]. *Drosophila* adults in experimental groups were treated for 3–5 days with a number of agents shown to increase misincorporation into protein or RNA, at doses leading to $<20\%$ mortality. Although these treatments produced error rates much higher than were seen during aging of control flies, the misincorporation rates subsequently returned to control (pre-treatment) levels, and the average lifespans of survivors were indistinguishable from controls [20]. Remarkably, this is exactly the prediction from our model. Since mortality follows first-order kinetics over time, the mortality increase from a stress depends only on the current value of the stress. Thus, the solutions of Eqs. (1)-(3) predict the same average lifespan of all animals surviving any stressful treatment as for controls (although the average lifespan of the entire treated group would obviously be shortened by inclusion of animals that died during treatment). Of course, stresses that induce appropriate defenses may cause hormesis, but this is beyond the intended scope of our simplified linear model.

Strategies for stabilization of genetic networks

Eq. (6) implies many possibilities to stabilize a regulatory network and thus extend lifespan, as summarized in Table I. According to Eq. (7), a reduction in genotoxic stress levels by isolation of genes from the environment can protect from direct stresses but can only produce a weak (logarithmic) increase in lifespan. Much stronger effects could be achieved by interventions aimed at increasing gene-network stability and hence reducing Λ . This can be accomplished, for example, if genes and their epigenetic states were isolated from regulatory signals, which equates to a decrease in β . This could be the reason why silencing of most signalling pathways – which initially involve kinase cascades but mostly terminate in transcription factors – accompanies extraordinary longevity and stress resistance in *C. elegans* [21, 22]. Such defensive strategies appear to have been utilized during the course of evolution, e.g., in protecting mitochondrial genes by their transfer to the nuclear genome, and by establishment of the nuclear envelope, considered a major factor leading to the emergence of multicellular life.

The relation between aging and the accumulation of defects is not limited to defects in the proteome, but can readily be extended to defects in the metabolome, including lipid metabolites, and the glycome (both of which are largely regulated by proteins, and thus under the indirect control of genes). It also encompasses Mobile Genetic Elements (MGE), genomic insertions of DNA ranging in size from hundreds to many thousands of base-pairs, interspersed throughout every mammalian genome (see a recent review [23]). Although derived from ancient DNA and RNA viruses, very few of these elements encode an active “transposase”, and most have accumulated too many mutations to be transposed (i.e., to jump within or between chromosomes) even in the presence of an active transposase, which may be transiently provided by an exogenous retrovirus. However, some MGEs are still mobile and thus highly mutagenic, either passively or actively. Being essentially defects in the transcriptome and interacting with the genome, this type of mutation fits our model very well. MGEs are abundant, covering approximately 30–50% of the human genome, and have been implicated as responsible for over 100 human genetic disorders including some cancers [24, 25]. Transposition of MGEs can be either cell-toxic or mutagenic, since they can disrupt essential genes or activate adjacent ones, so they may account for a significant proportion of spontaneous genome damage. Considering that MGEs evidently evolved from viruses, it is not surprising that equations similar to Eqs. (1)-(3) and the stability criterion similar to (6) have been identified to describe infectious disease dynamics and stability of the virus-host system [26, 27]. The hypothesis that MGEs play a significant role in mutagenesis and genomic instability predicts that lifespan may be increased by strategies aimed at reducing either MGE transposition or recombination

The model parameter	Biological embodiment
Coupling rate, β	Protective nuclear wall; transfer of mitochondrial genes to the nuclear genome; inhibition of MGE(RT)
“Effective” genome size, G	Epigenetic inactivation of genes; tissue differentiation and specialization; temporal restriction of gene expression
Expressome (proteome, metabolome) turnover rate, c	Turnover/repair of proteins and metabolites: their dilution via cell division, asymmetric division; chaperones, proteasomes, and autophagosomes; metabolome turnover/maintenance; apoptosis (in multi-celled organisms)
DNA repair rate, δ	DNA repair; defenses against viruses and mobile genetic elements
Genotoxic stress level, f	Isolation from environment; suppression of ROS; dietary preferences and avoidance of noxious biomaterials; development of nociceptors and learned responses

Table I: Possible lifespan extension strategies with relation to the gene network stability model parameters, and possible examples of their evolutionary deployment.

rates, p or β , by inhibiting the enzymes such as transposases and reverse transcriptases (RTs), which mediate their mobility. This prediction has been validated for at least some species [28].

Turnover of the proteome or metabolome, c , and repair efficiency, δ , are factors shown to modulate lifespan in several species. Indeed, DNA repair pathways are encoded by hundreds of genes [29], that are variously involved in detection of DNA damage, enzymatic manipulation of damaged DNA, and homologous recombination between DNA strands, often permitting complete restoration of the original sequence even when portions have been lost from one chromatid or chromosome homolog [23]. A somewhat more surprising result is that both the proteome and genome repair rates, c and δ respectively, formally contribute to the result on an equal footing. This means that increasing protein turnover may help protect against DNA damage and *vice versa*. Increased protein turnover rates, as can result from increased ubiquitin-proteasome activity, have been demonstrated to result in increased longevity in yeast. Enhanced proteasomal activity confers a 70% increase in median and maximum replicative lifespan, comparable to the longest lived single gene deletions identified, and greater than the extension observed by deletion of TOR1 or over-expression of SIR2 [30].

Recent data reveal that the NMR has highly efficient protein degrading machinery and thereby maintains high levels of protein quality control, constantly degrading misfolded and damaged proteins, thus maintaining uniform steady-state levels throughout life [31]. Our model predicts that DNA repair efficiency, δ , is as important for lifespan extension as increases in the protein turnover rate, c . These NMR results are interesting because their exceptional longevity occurs despite the presence of chronic oxidative stress even at young ages [8, 32]. Remarkably the stability of the proteome and its relationship to lifespan were previously analyzed in a related

context [33] with very similar conclusions.

Cell division is a trivial way to rebuild the cell components and dilute expressome defects in half (symmetric division) or more (asymmetric division), especially relevant to yeast and continuously dividing tissues. This simple dilution principle links the protein turnover rate to cell division frequency, so that decreasing the protein turnover rate c , by inhibiting cell-cycling or by other means, may be used as a gene-network destabilization strategy for anti-cancer and antibiotic treatments [34].

In differentiated organisms there appear even more ways to maintain stability of the gene network. Metazoan (multi-tissued) animals have the ability to eliminate cells that have sufficiently damaged or unstable genomes, in a process called apoptosis, and replace them with healthy, stem cell-derived cells. Thus the repair rates c and δ also cover the contributions of these apoptotic and regeneration pathways. Moreover, as cells divide and differentiate to form new tissues and cell types, resulting in many different epigenetically stable states of the same genome, all of the model constants can also vary depending on the tissue involved. Thus our model clearly permits the instability and aging rates of different tissues to vary. This does not, however, alter the outcomes, which will reflect the vulnerability of the most unstable tissue on which animal survival depends – most probably, the stem-cell subsystem corresponding to the most renewal-dependent tissue within the body.

According to the stability requirement (6), large genomes are difficult to maintain. There are multiple ways to increase system stability by keeping the size of the expressed genome under control. One such strategy, clearly of ancient origin, is differentiation, wherein only a small fraction of the full genome is expressed by any one cell type at any point in time. Another possible way to regulate the stability of the genetic network is by modulating the degree of network connectivity. The robustness of a simulated gene network with respect to

external noise was recently shown to be associated with the connectivity of the network [35]. This compares very well with our stability condition Eq.(6), which predicts that a gene network becomes stable if the characteristic measure of network connectivity becomes sufficiently small: $K < K_0 = c\delta/(p\beta G)$. A recent study of long-lived *Myotis brandtii* genomes found a wide range of genetic abnormalities in the GH/IGF1 axis [36]. Ablation of growth hormone signaling may produce a reduction of the effective network connectivity K , which thus could be one of several possible explanations for the extreme life expectancy of long-lived bats. All the above strategies are mathematically equivalent and exist in nature; indeed, there are cases in which multiple strategies are employed to increase lifespan [36].

Conclusions and Prospects

In summary, we have provided a mathematical explanation for the dramatic variance in lifespans seen in the animal kingdom, relating this variance to genetic-network stability and resistance to stresses. We developed a model, which lets us define Gompertzian or “normal” aging as an exponential accumulation of gene-regulation abnormalities rooted in the inherent instability of gene networks occurring under most common circumstances, and causing a progressive loss of stress resistance with age. This, in turn, produces susceptibility to age-related diseases (or may even cause their onset), and leads to death of an organism. This seems consistent with previously reported experimental mouse data indicating that epigenetic dysregulation contributes far more (by up to two orders of magnitude) to the loss of gene-expression integrity than somatic mutations alone [37]. On the other hand, gene-networks of animals with better transcription fidelity or other mechanisms of genome-maintenance are not only more stress-resistant, but under specific conditions may become stable and produce negligible senescence phenotype [38]. An even more im-

portant corollary is that the gene networks of extremely stress-resistant animals (extremophiles) can still be unstable, because network stability is determined by the value of the combined parameter R_0 given by Eq. (6), and not directly by high c and δ rates.

The most important results of this study are Eq. (4), phenomenologically describing aging of a gene network, and the concept of fundamental genomic instability described by Eq. (6). We show that the lifespan of a species is determined by the stability of its most vulnerable gene network, coupled with its expressome in a realistic environment. Mathematically, there exist two types of solutions to Eq. (4), implying the possibility of both stable and unstable phases. We posit that the stable phase corresponds to negligible senescence and is robust with respect to environmental or endogenous noise. Although unstable gene networks exponentially accumulate gene expression errors and eventually disintegrate, the growth exponent is nevertheless small. We believe that Eq. (6) represents the same phase boundary previously identified in [39]. The critical dynamics of gene networks is a natural way to bridge the “hierarchy of scales” problem, which is to explain how lifespans can greatly exceed the time scales characterizing any of the constituent processes in living cells. The genetic networks of most animals are inherently unstable and this leads to their aging. Since stabilization of gene networks can be favored in multiple ways, further research has the clear potential to create novel therapies to protect against the most morbid age-associated diseases, and perhaps even against aging itself.

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